

CLAIMS:

1. A method of assaying for folate in a folate containing sample, wherein at least some of said folate comprises at least one attached glutamate residue, said method comprising:

subjecting said sample to hydrolysis to release paraaminobenzoic acid, p-aminobenzoyl glutamic acid, or a salt thereof; contacting the released paraaminobenzoic acid, p-aminobenzoyl glutamic acid, salt, or a diazo derivative thereof, with a binding partner therefor; and directly or indirectly detecting the resulting binding partner:paraaminobenzoic acid, binding partner:p-aminobenzoyl glutamic acid, or salt or derivative combination.

2. A method as claimed in claim 1 wherein said method does not comprise any chromatographic separation steps.

3. A method as claimed in claim 1 or claim 2 wherein said sample is a blood derived sample.

4. A method as claimed in any of claims 1 to 3 wherein said binding partner is selected from an antibody, an antibody fragment, a single chain antibody, a single chain antibody fragment, an oligopeptide, an oligonucleotide and a small organic molecule.

5. A method as claimed in claim 5 wherein said small organic molecule is an aromatic tertiary amine, phenol or phenol derivative capable of forming a diazo compound with paradiazobenzoic acid (PDBA) or paradiazobenzoyl glutamate (PDBA-glu).

6. A method as claimed in any of claims 1 to 5 wherein said hydrolysis comprises treating said sample with a metal catalyst under acidic conditions.

REPLACED BY  
ART 34 AMDT

- 21 -

7. A method as claimed in any of claims 1 to 6 wherein said hydrolysis comprises treating said sample with microwave radiation.

8. A method as claimed in any of claims 1 to 7 wherein said hydrolysis comprises treatment with an oxidising agent.

9. A method as claimed in claim 8 wherein the oxidising agent is hydrogen peroxide and/or potassium permanganate.

10. A method as claimed in any of claims 1 to 9 wherein said hydrolysis comprises treatment with a reducing agent.

11. A method as claimed in claim 10 wherein the reducing agent is sodium borohydride.

12. A method as claimed in any of claims 1 to 11 wherein said hydrolysis comprises oxidative photolysis.

13. A method as claimed in claim 12 wherein said oxidative photolysis is carried out in the presence of a photosensitiser.

14. A method as claimed in any of claims 1 to 13 wherein said sample is incubated in the presence of naturally occurring and/or added enzymes whereby to remove all but the terminal glutamate residue from said folate and wherein the product of the hydrolysis is PABA-glu.

15. A method as claimed in any of claims 1 to 13 wherein said sample is incubated in the presence of at least one added enzyme, whereby to remove all glutamate residues from said folate, and wherein the product of

REPLACED BY  
ART 34 AMDT

- 22 -

the hydrolysis is PABA.

16. A method as claimed in any of claims 1 to 15 wherein said binding partner: paraaminobenzoic acid, binding partner: p-aminobenzoyl glutamic acid, or salt or derivative combination is detected directly by absorbance or fluorescence.

17. A method as claimed in any of claims 1 to 15 wherein said binding partner: paraaminobenzoic acid, binding partner: p-aminobenzoyl glutamic acid, or salt or derivative combination is detected indirectly by means of a secondary binding partner.

18. A kit for use in the performance of the assay of the invention, said kit comprising:

- i) a folate hydrolysis reagent; and
- ii) a PABA, PABA-glu, PDBA or PDBA-glu binding partner;

19. A kit as claimed in claim 18 additionally comprising an enzyme or enzyme cocktail.

20. A kit as claimed in claim 18 or claim 19 additionally comprising a PABA to PDBA or PABA-glu to PDBA-glu converting reagent.

21. A kit as claimed in any of claims 18 to 20 additionally comprising a secondary binding partner.

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ART 34 AMDT